

TRANSPORT OF RADIOACTIVE AUXINS IN THE SOUTH AFRICAN LABIATE, *IBOZA RIPARIA*¹

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ABSTRACT

Transport of the auxins, IAA, NAA and 2,4-D, was studied in stem sections of staminate and pistillate *Iboza riparia* and was found to be affected by sex, age and development of flowers. In young shoots, transport was rapid with a very short time delay prior to auxin efflux from the shoot sections. In older shoots, auxin efflux was retarded by several hours. The rate of transport was lowest at the time of flowering but recovered somewhat during subsequent weeks. Under some conditions the sexes differ appreciably in their ability to transport auxin.

UITTREKSEL

VERVOER VAN RADIOAKTIEWE OUKSIEN IN *IBOZA RIPARIA*, 'N SUID-AFRIKAANSE LID VAN DIE LABIATAE

Die vervoer van die ouksiene indool-3-asynsuur (IAS), naftalienasynsuur (NAS) en 2,4-dichlorofenoksiasynsuur (2,4-D) is in stingelseksies van meeldraad- en stamperdraende *Iboza riparia* bestudeer; dit is gevind dat dit deur geslag, ouderdom en ontwikkeling van die blomme beïnvloed word. Die vervoer in jong stingels is betreklik snel met slegs 'n baie kort tydvertraging voor uitskeiding van ouksien uit die stingelseksies. In ouer stingels, daarenteen, is ouksien-uitskeiding met 'n aantal ure vertraag. Die snelheid van vervoer is laagste tydens blomvorming maar neem in die daaropvolgende weke weer toe. Onder sekere toestande verskil die geslagte aanmerklik in hulle vermoë om ouksien te vervoer.

INTRODUCTION

Studies of auxin transport have progressed from qualitative descriptions of velocity, polarity and distribution in organs and whole plants to quantitative studies on kinetics and interactions of growth hormones and inhibitors, co-factors and conjugates involved in movement and metabolism of both endogenous and synthetic auxins (Fawcett, 1961; Kaldewey, 1967; Goldsmith, 1968, 1969; Thimann, 1969). Much of the present knowledge of auxin movement and metabolism was elucidated by work with *Coleus* (Jacobs, 1965, 1967), *Phaseolus* (Jaworski, Fang and Freed, 1955; McCready, 1963, 1968; McCready and Jacobs, 1963 a,b), *Helianthus* (de la Fuente and Leopold, 1970 a,b) and *Zea* (Scott and Wilkins, 1968, 1969; Wilkins and Scott, 1968).

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We decided to conduct our experiments with a South African labiate, *Iboza riparia*, and to give attention principally to questions of (a) changes in auxin transport during the period of an experiment, (b) the influence of shoot age on transport, and (c) the influence of shoot sex on transport.

There were several reasons for the choice of *Iboza*. The plant is related to *Coleus*, dioecious, readily propagated, and resistant to desiccation. *Iboza* shows conspicuous morphological differences between the staminate (male) and pistillate (female) plants. For example, stems of female plants are more glabrous than those of male plants, female plants also mature (flower) sooner than the males and their leaves are smaller and darker. However, examination of stem sections disclosed no internal anatomical difference between the sexes. The staminate flowers are somewhat larger than the pistillate. The flowers of both sexes are very small and pale lilac in colour and calyces are minute and campanulate in the two sexes. The ovary is four-lobed but is abortive in the staminate flower. The stamens number four; they are absent in the pistillate flower.

MATERIALS AND METHODS

Nine-cm long slips from clones of male and female *Iboza riparia* NE. Br. shrubs were grown into plants each with five to six internodes. All plants were topped at this stage to promote lateral shoot development and experiments were done with fourth internodes of lateral shoots, counting from the apex downwards. Each experiment was conducted with 15 mm sections from the same internode or, if that was not possible due to short internode length, a similar fourth internode from another shoot of the same plant was used, thus eliminating any minor physiological differences that might occur from plant to plant.

Agar block technique

The 15 mm sections were cut and placed in petri dishes on $5 \times 5 \times 2$ mm receivers of 3% w/v agar. Donor blocks were placed on top of the upright sections and a 10 μ l droplet of IAA-1- 14 C applied to each of the donors. At the termination of experiments, donor and receiver blocks were removed and placed in numbered scintillation vials, to which 2.0 ml of absolute ethanol had previously been added. When so required, sections were frozen and cut into 12 equal transverse subsections on a freezing microtome. The serial subsections were placed in scintillation vials containing 2.0 ml of absolute ethanol, ground finely with a glass rod, and 15.0 ml of Bray's (1960) liquid scintillation medium added. Donor and receiver blocks also were broken up with glass rods and 15.0 ml of Bray's medium added to the respective vials. Samples were counted for 10 minutes at 0°C in a Unilux II liquid scintillation spectrometer. Absolute dpm values were calculated on an Olivetti Programma computer.

U-Tube technique (Fig. A)

In preliminary experiments using agar blocks it was noticed that the amount of auxin obtained in receiver blocks fell off rapidly with shoot age, especially in female shoots. Since this effect might have been due to increased oxidative inactivation of auxin at the cut surfaces, it was thought that if the donor and receiver systems were aqueous, oxygen would be more effectively excluded from the cut surfaces. Consequently a U-tube apparatus was devised in which aqueous solutions at equal pressures could be maintained at each end of a shoot section. Donor and receiver parts were identical and consisted of L-shaped pieces of Pyrex glass tubing. Tubes with several internal diameters (from 4,5 to 7,0 mm) were made to accommodate stem sections of corresponding diameters. Long arms of the apparatus were ca. 5 cm and held upright; short arms were ca. 1 cm and held horizontally with the shoot sections sealed to and bridging the two short arms. The columns of donor and receiver solutions were kept at the same height.

Prior to cutting and waxing the stem section was blotted on tissue paper and then sealed in position with a low melting-point paraffin wax (40°C). Glass distilled water was pipetted into donor and receiver arms of the apparatus, care being taken that levels in donor and receiver arms were identical. The system was checked for leaks and allowed to equilibrate for 0,5 h prior to the addition of IAA-1-¹⁴C to the donor arm.

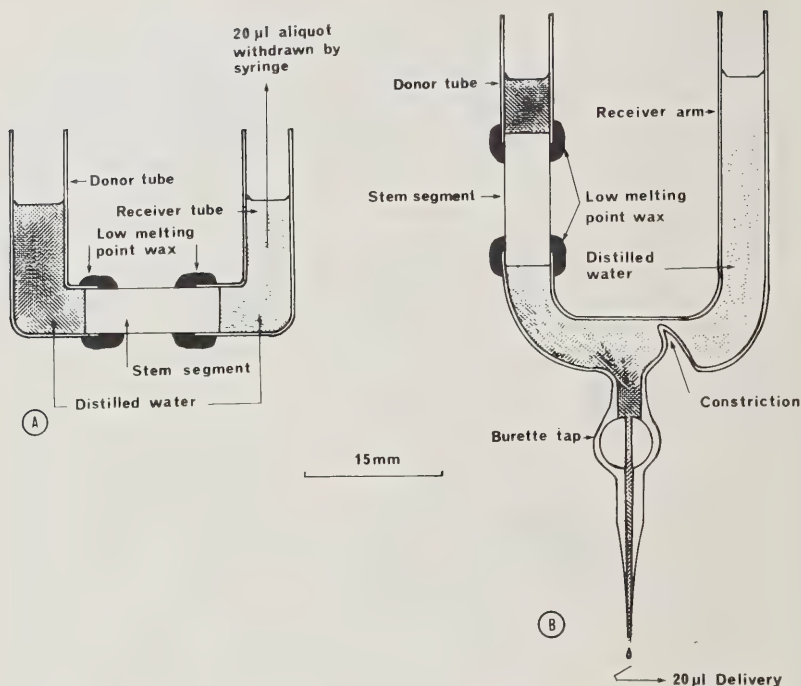
At preselected time intervals, a 200 µl sample was withdrawn from the receiver with a tuberculin syringe and re-injected rapidly, effectively mixing the auxin and the water. This process was repeated four times, after which a 20 µl sample was withdrawn from the bottom of the receiver with a 500 µl glass syringe. The 20 µl samples were dispersed into coded scintillation vials to which 15,0 ml of Bray's medium was subsequently added. Samples were counted and computed as described above. After withdrawal of each sample, 20 µl of glass-distilled water was added to the receiver to equalise the heights and therefore the pressures of the water columns.

Ringbarking technique

To obtain an indication of the amount of auxin moving in the xylem relative to that moving in the phloem and cortical layers, stem sections were ringbarked by excising a 2 mm wide ring of tissue 5 mm from either the apical or the basal end of the section. Care was taken to remove only the phloem and cortex and to expose the vascular cambium. The exposed tissues were then coated with paraffin wax and U-tube arms attached as described above.

Microburette technique (Fig. B)

To facilitate frequent sampling during the longer experiments the receiver arm of the U-tube apparatus was modified into a kind of microburette. This



FIGS. A and B.

Diagrammatic representation of U-Tube (Fig. A) and Microburette (Fig. B) set-ups used to determine radioactivity in aqueous samples.

was also a U-shaped vessel held upright and having a burette tap at the base between the two arms. The shoot section was waxed in place on the short arm and a short donor tube was waxed in place above the upright shoot section. The longer arm was filled with water to a level equal to that of the liquid in the donor tube.

Assembled microburettes were clamped to retort stands, the donor tube and receiver arm were filled with glass-distilled water, and the water levels equalised. A 0.5 h equilibration period was allowed prior to the application of a calculated volume of radioactive IAA, NAA or 2,4-D which, on assay, yielded equal dpm values.

The collection technique was as follows: 200 µl, ca. 10 droplets, were run out of each burette prior to collection of a 20 µl sample, i.e. the 11th drop, for liquid scintillation assay. The volume of the capillary tube above the

burette tap was designed to be approximately 200 μl . Collection of the 200 μl in a waste beaker reduced the sample numbers per experiment to one tenth of what otherwise would have been required, and still yielded a satisfactory estimate of the volume of ^{14}C in the burette and hence the rate of transport of the labelled auxin.

Auxin solutions

Labelled 3-indoleacetic acid-1- ^{14}C (IAA-1- ^{14}C), naph-1 yl-acetic acid-1- ^{14}C (NAA-1- ^{14}C), and 2,4-dichlorophenoxyacetic acid-2- ^{14}C (2,4-D-2- ^{14}C) were purchased from the Radiochemical Centre, Amersham.

The specific activities were as follows:

- IAA-1- ^{14}C : 57 mCi/mM (322 $\mu\text{Ci/mg}$),
- NAA-1- ^{14}C : 50,6 mCi/mM (270 $\mu\text{Ci/mg}$),
- 2,4-D-2- ^{14}C : 29 mCi/mM (131 $\mu\text{Ci/mg}$).

The benzene solvents were removed under vacuum and the recrystallized auxins dissolved in absolute ethanol and diluted to 4 ml with sterile water (water-ethanol, 3:1 v/v). The stock solutions were kept in sterile, foil-wrapped injection ampules, and at no stage exposed to bright light. One ml of each of the solutions was withdrawn from the stock ampules with clean 1,0 ml glass tuberculin syringes, injected into sterile 10 ml rubber-stoppered ampules and diluted with glass-distilled water: absolute ethanol (9:1, v/v) to give approximately equal dpm values. Standards containing 10 μl of the diluted ^{14}C -labelled auxins were then withdrawn, injected into scintillation vials and 15,0 ml of Bray's scintillation medium added. Prepared samples were counted at 0°C and the results computed to an error of not more than 0,05%. The specific activities of the prepared samples were as follows:

- IAA : 2380,05 dpm/ μl (0,0322 $\mu\text{g}/\mu\text{l}$),
- NAA : 3544,95 dpm/ μl (0,0270 $\mu\text{g}/\mu\text{l}$),
- 2,4-D : 3836,21 dpm/ μl (0,0131 $\mu\text{g}/\mu\text{l}$).

Computer technique

An Olivetti Programma P-101 was utilized to facilitate rapid calculation of the dpm per sample and the standard deviation of the count. A preset counting time of 20 min usually yielded a counting error of less than 1,5% in our experiments. In the initial experiments, in which finely ground tissue was used, the quenching effect of the tissue was determined and corrected for by computer.

RESULTS

Experiments using donor and receiver agar blocks

The first experiment was designed to give a general picture of the transport and distribution of applied auxin in an *Iboza* internode section over a 24 h

period. Sections from the fourth internode of 2-week old shoots of male *Iboza* were used in this experiment. The distribution of radioactivity was determined after 0,5; 1,0; 1,5; 7,0 and 24,0 h by cutting the original 15 mm sections into 12 equal subsections. Each part of the experiment was carried out in triplicate. The principal results are shown in Fig. 1 and Table 1. Results for the distribution of ^{14}C were plotted separately per time interval and irregularities in the curves smoothed out as follows: the mean activity recorded in subsections 1 & 2, 2 & 3, 3 & 4, and so on to 11 & 12, were totalled and the midpoint between each pair of these plotted, the nett result being that in the three dimensional figure all points are represented.

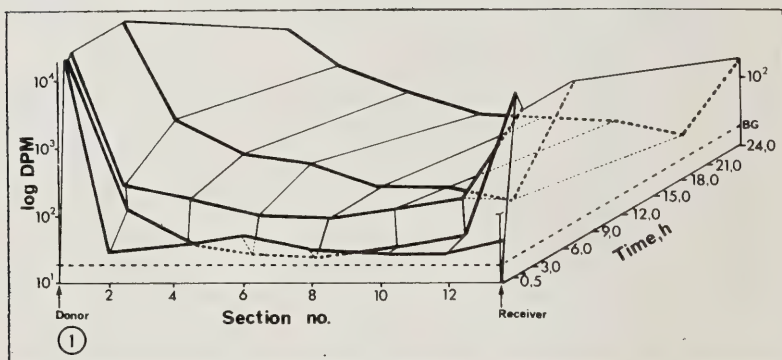


FIG. 1.

Basipetal distribution of radioactivity from IAA in 2-week male *Iboza* internode sections. Radioactivity was measured over a 24 h period in donor blocks, in receiver blocks and in 12 tissue subsections. The abscissae are plotted on a logarithmic scale. Note accumulation in the apical subsections and initial rapid transport indicated by high activity in receiver blocks with low activity in the central subsections. Accumulation of radioactivity within the section as a whole was appreciable after 1,5 h. Note also the gradual decline of activity in receiver blocks from 6 h onwards. BG, background count.

The data show an initial rapid movement of radioactivity throughout the section and into the receiver, the distribution being almost equal after 0,5 h. By the end of 1,0 h there was an appreciable accumulation in the receiver and an indication of accumulation in the apical subsections. By 1,5 h, ^{14}C accumulation in the section had increased markedly, but activity in the receiver had fallen from 7887 dpm at 1,0 h to 3980 dpm at 1,5 h. Radioactivity accumulated in the apical subsections up to 7 h but declined after that. Radioactivity in basal subsections declined after 1,5 h, whilst in receivers activity continued to increase from 3980 dpm at 1,5 h to 9946 dpm at 24 h.

TABLE I.
Distribution of basipetally-translocated radio-activity from IAA-1-¹⁴C in 15 mm sections from fourth internodes of 2-week male *Iboza* shoots.

Time ,h	DPM					Total
	Donor	Subsection number			Receiver	
		1	6	12		
0,5	28 332	57	60	50	62	28 561
1,0	21 460	189	28	58	7887	29 622
1,5	23 312	280	84	175	3980	27 831
7,0	21 157	1592	63	35	4977	27 824
24,0	17 340	890	46	49	9946	28 271

U-tube experiments

In these experiments sections from male and female shoots 4,5; 9,5 and 11,0 weeks old were used. Radioactive IAA was placed in the donor solution and the receiver solution was sampled at five-minute intervals up to 30 min. The results are summarised in Figs. 2 and 3 and Table 2. The most noteworthy feature of these results is the decline in transport with shoot age; with 11-week sections the rate was about one-third of what it was with the 4,5-week sections. Total counts for the male sections was 1159 dpm compared with 1173 dpm for the samples from the female sections. Table 2 indicates that by 9,5 weeks the total radioactivity recovered from the male shoot sections had dropped to 54% and the female to 61% of that recovered from male and female shoot sections aged 4,5 weeks, respectively. In shoot sections aged 11,0 weeks, the total radioactivity recovered had decreased to 33% of the 4,5 week totals. There seems to be an indication that the overall transport of auxin decreases with time more rapidly in the male shoot sections than the female, but it is evident that the efflux of auxin was more rapid in the male than the female shoot sections.

Thus there was a small but probably significant tendency for the male tissues to transport auxin more rapidly than the female tissues. This tendency is more clearly manifest in the results shown in Figs. 4 and 5 where the male sections have obviously transported more auxin than the female sections.

Comparison of IAA transport in intact and ringbarked male and female explants

In their studies of auxin transport in *Coleus*, Jacobs and McCready (1967) noted that there was no significant difference between the rate of transport by pith, and the rate by corner tissues which contain the xylem and phloem, even though the corner tissues had fewer, and larger cells in cross-section. We

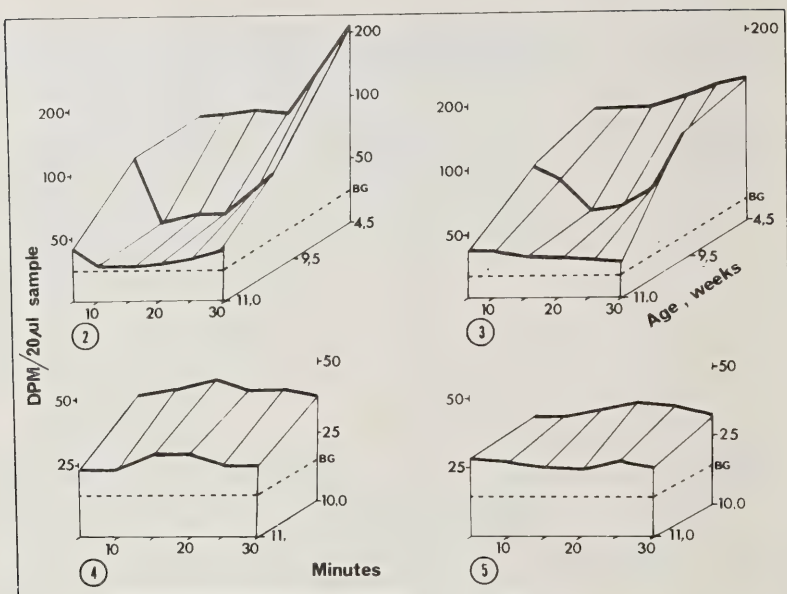


FIG. 2.

Time course of basipetal transport of IAA in male shoot sections as measured by the manometric method during a 30 minute period. The sections were taken from shoots 4,5; 9,5; and 11,0 weeks old. Note rapid decline in auxin transport with increasing shoot age. Data represent mean of six replicates. BG, background count.

FIG. 3.

Time course of basipetal transport of IAA in sections from female shoots aged 4,5; 9,5 and 11,0 weeks. Again note the rapid decline in the ability of the tissue to transport auxin with increasing shoot age. BG, background count.

FIG. 4.

Time course of transport of IAA in ringbarked sections from male shoots aged 10,0 and 11,0 weeks. Data represents mean of 10 sections. BG, background count.

FIG. 5.

As per Fig. 4 except that sections were from female shoots. Note that auxin transport through ringbarked female sections was lower than through ringbarked male sections. BG, background count.

were interested in conducting a related experiment with *Iboza* to determine if the aqueous donor and receiver system of the glass tubes might in some way permit a rapid movement through the xylem and thus lead to inaccurate interpretations of the transport system of intact plants. In this experiment sections from 10 and 11-week old male and female shoots were ringbarked 5 mm from

TABLE 2.
Accumulation of basipetally-translocated IAA-1-¹⁴C in receivers in 15 mm maleⁱ and femaleⁱⁱ shoot sections (from fourth internodes) of *Iboza*.

Age (weeks)	DPM		% DPM	
	Male	Female	Male	Female
4,5	615	603	100	100
9,5	338	371	54	61
11,0	206	199	33	33
Total	1159	1173		

i 23 replicates.

ii 21 replicates.

either the apical or basal ends. Transport of IAA was followed by the U-tube technique for a 30 minute period. The results are summarised in Figs. 4 and 5. No difference was found between the two positions of ringbarking; the points in the figures represent means of replicates of both positions. There was little change in transport during the course of this experiment. However the male sections transported appreciably more IAA than did the female sections.

To determine the relative efficiency of transport in the xylem/pith compared with the phloem/cortex, cross-sectional areas of twenty sections were measured. The relative areas are shown in Table 3: the xylem/pith area comprised 64% of the total. In the same table are shown the 30 min transport rates of the 10-week male and female sections. (Data from the 11-week sections were not included because the counts were very close to background levels.) The table shows that although the xylem/pith comprised 64% of the total area, those tissues transported only 44% in the male and 33% in the female of the auxin (transported by intact control sections). Thus the phloem/cortex appears to be considerably more efficient in transporting auxin than does the xylem/pith. The foregoing conclusion presumes that any wound effects were relatively minor as the experiment itself was not designed to exclude completely that possibility. However, from the work of others (e.g., Jacobs and McCready, 1967) such wound effects would appear to be small. For the present it is not possible to account for the seeming difference between our results, which showed much greater transport by phloem/cortex than by xylem/pith, and those of Jacobs and McCready (1967) which showed the same rate of transport by pith as by corner (xylem/phloem) tissues. Probably the explanation lies somewhere among the several differences in materials and methods employed in the two sets of experiments.

TABLE 3.
The effect of ringbarking on the rate of transport of IAA-1- ^{14}C in 10-week male and female *Iboza* shoot sections after a 30-min transport period.

	Relative cross-sectional area (%)	DPM		% DPM	
		Male	Female	Male	Female
Control (intact)	100	80	90	100	100
Xylem and pitch (ringbarked) . . .	64	35	30	44	33
Phloem and cortex (by difference) . . .	36	45	60	56	67

Microburette experiments

To gain further information on the decreased IAA transport in sections from older shoots the specially designed microburettes were used and the monitoring time was increased. The results of the first experiments, with sections from male and female shoots 12,5 weeks old and both basipetal and acropetal transport are shown in Fig. 6. They indicate that auxin transport is not a continuous, steady-state phenomenon, but consists of a phase of tissue loading, followed by a phase of increased auxin efflux up to a maximum (ΔT_{max}), and a sharply defined lag phase of transport during which the level of detectable ^{14}C from the auxin decreased. With the female section, detectable ^{14}C , although initially low (28 dpm) increased to 100 dpm at 1 h. Efflux of ^{14}C remained static at ca. 100 dpm from 1 h to 3,5 h; thereafter the efflux increased rapidly to a ΔT_{max} at 5 h of 140 dpm. With the male section, auxin efflux increased gradually from 38 dpm at 0,5 h to 48 dpm at 0,8 h and to 80 dpm at 2,0 h. Maximum efflux, 185 dpm, was attained at 4,25 h. Thus in basipetal transport, the female differed from the male section in having a shorter tissue loading phase, a lower ΔT_{max} , and less total transport.

With acropetal transport ΔT_{max} was not sharply defined for either sex. Both curves showed a low plateau of transport of about 2 hours. However the data suggest that the transport rate falls off earlier in the female than in the male. With young, 3-week sections (Fig. 7), there was little evidence of a tissue loading phase. Maximum efflux, 1350 dpm, was reached at 3,0 h followed shortly by a lag phase with a ten-fold decrease. In contrast the sections from 15-week shoots showed little change in transport during the eight-hour experimental period and little difference between the acropetal and basipetal directions of transport.

Comparisons of IAA, NAA and 2,4-D transport in male and female stem sections

At this stage sufficient evidence was at hand to justify the investigation of synthetic auxins. Experiments on basipetal transport of IAA-1- ^{14}C , NAA-1- ^{14}C

and 2,4-D-2-¹⁴C through male and female sections aged 17 and 22 weeks were conducted using the microburette technique. The results are summarised in Figs. 8–11.

Correlated with visible development of the inflorescence in female plants, auxin efflux changed markedly between weeks 15 and 17 (Figs. 7, 9). At week 15 basipetal transport in the female section was essentially similar to acropetal transport in the male (Fig. 7). However, by week 17 basipetal transport of IAA in both males and females attained ΔT_{maxima} by 8 h after time delay periods of from 4 to 6 h (Figs. 8 and 9).

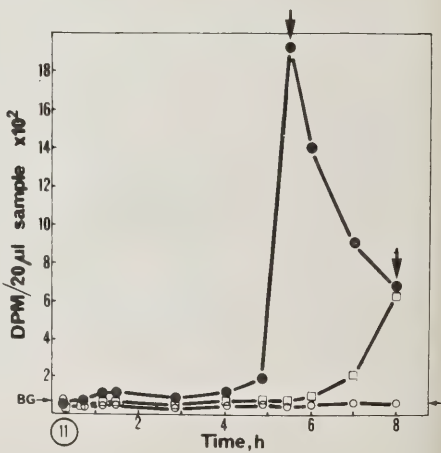
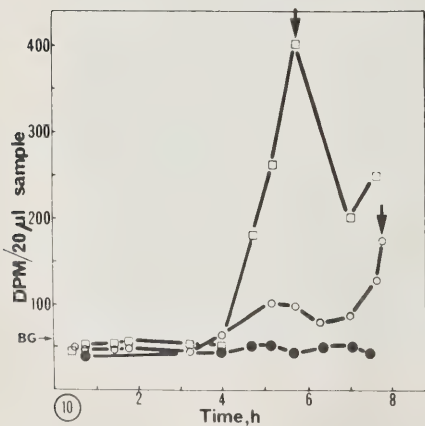
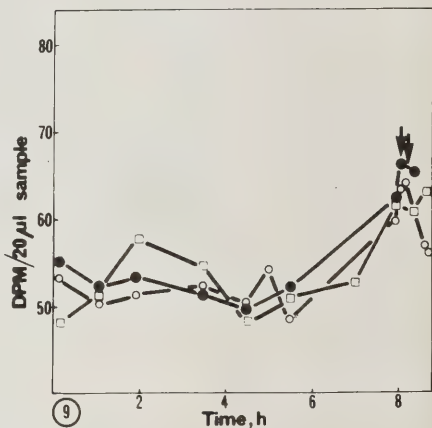
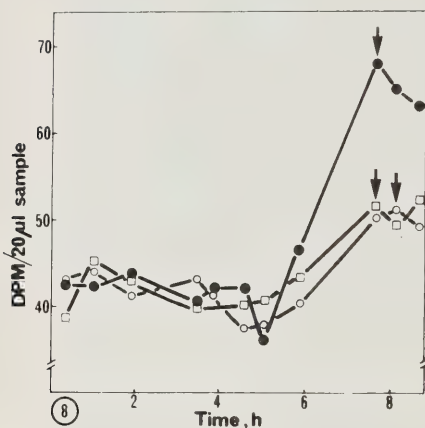
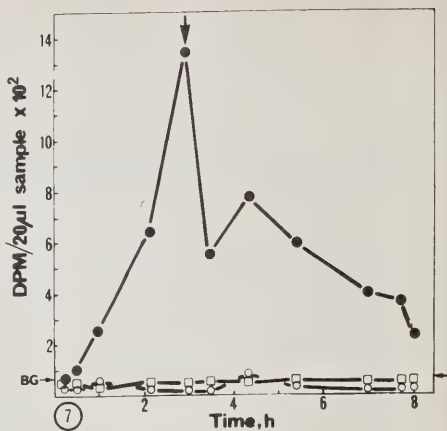
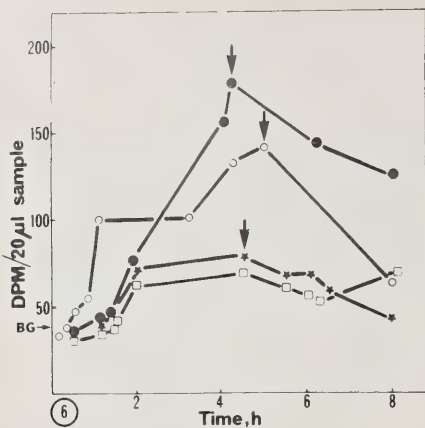
The transport patterns of IAA and 2,4-D showed similar trends (Figs. 8 and 9); however 2,4-D was transported to a greater extent than either NAA or IAA in both male and female sections.

Between week 17 and week 22 formation of flowers by female plants ceased, and correlated with this was a marked increase in the rate of auxin transport, more so in female than in male sections (Figs. 10 and 11). Of interest is the difference between male and female sections in the rate of transport of radioactivity from 2,4-D. In the male section transport remained static at ca. 50 dpm/sample. In the corresponding female section, 2,4-D transport increased to 110 dpm at 1.5 h, and after 4 h rose steeply to a ΔT_{max} at 5.5 h of 1897 dpm. Both male and female sections showed a prolonged tissue loading phase with IAA and NAA. In the male sections transport of both these auxins reached maxima shortly after 5 h, then declined, and then rose again and was still rising at the termination of the experiment. In the female sections transport of IAA and NAA did not reach maxima but the rates were rising sharply at termination.

DISCUSSION

The results of the experiments with *Iboza* stem sections on the uptake, distribution and efflux into receivers of radioactivity from IAA-1-¹⁴C, NAA-1-¹⁴C and 2,4-D-2-¹⁴C indicate that auxin transport is NOT a steady-state phenomenon in this plant. Figure 1 showed that between 1.0 and 1.5 h radioactivity from IAA increased and reached a maximum in the section at 7.0 h, and then declined. Rapid accumulation in the receiver during the first hour, coupled with low activity in stem sections is probably the result of a high rate of transport. The decrease in receiver activity during the period 1.0 to 1.5 h suggests that a major portion of the transportable auxin pulse has, by this time, moved out of the tissue section and that accumulation of IAA or its metabolites has commenced. Maximum accumulation of IAA or its metabolites within the apical portions of the shoot sections occurred between 1.5 and 7.0 h.

The effect of shoot age on the decline in auxin transport is demonstrated in Figs. 2, 3, 6 and 7. The data of Figs. 6–11 show that the time taken to pre-



load the sections with exogenous auxin prior to efflux, increases with shoot age. The reasons for the decrease in basipetal transport of auxin with shoot age are not clear. Possibly there is a decrease in the availability of transport sites within the section as a consequence of ageing or of the physiological changes associated with flower formation.

Naqvi and Gordon (1965) with *Coleus* found a decrease in the ratio of basipetal to acropetal transport from 3.0 to 1.3 in non-flowering and flowering plants. Our results also showed that flowering affects the rate of transport of auxin (see Figs. 6–11). Although flowers were not formed in male plants, suppression of auxin efflux was most marked at week 17, the time of female flowering (Fig. 8). In 22-week female shoots (Fig. 11) flower formation had ceased and this was marked by a strong rise in radioactive efflux. Similar results were obtained in 22-week male sections (Fig. 10).

The microburette technique proved very convenient for determining the efflux of auxins from stem sections. Experiments using this technique showed that the time-delay factor (Kaldewey, 1967) increased with shoot age. With *Iboza* in most instances, an increased transport of radioactivity followed the

FIG. 6.

Transport of radioactivity from IAA in sections from male and female shoots aged 12.5 weeks, indicated as follows: basipetal, male (●—●) and female (0—0); and acropetal, male (□—□) and female (*—*). BG, background count. Note that basipetal transport rate in male sections is higher than in the corresponding female sections. ↓, $\Delta^T \text{max}$.

FIG. 7.

Transport of radioactivity from IAA in sections from 3.0 week male shoots and 15.0 week male and female shoots, indicated as follows: basipetal, 3-week male (●—●); acropetal 15-week male (0—0); and basipetal, 15-week female (□—□). BG, background count. Basipetal transport in sections from 3-week male shoots increased after 0.28 h, whereas no real change in transport was found with either the 15-week male or female shoots. A 10-fold decrease in activity occurred after $\Delta^T \text{max}$ in 3-week male shoots.

FIG. 8.

Comparison of basipetal transport of IAA (0—0), NAA (□—□) and 2,4-D (●—●) in sections from male shoots aged 17 weeks. Note higher rate of transport of 2,4-D and the longer time delay from 0.28 h (Fig. 7) to 5.0 h (this Fig.), prior to detection of significant increases in auxin efflux. Background not inserted due to low activity of the samples.

FIG. 9.

Comparison of basipetal transport of IAA, NAA and 2,4-D in sections from female shoots 17 weeks old. Symbols as for Fig. 8. Note the very long time delay prior to an increase in ^{14}C -efflux and the attainment of $\Delta^T \text{max}$. Background not inserted due to very low activity of the samples.

FIG. 10.

Comparison of basipetal transport of IAA, NAA and 2,4-D sections from male shoots 22 weeks old. Symbols as in Fig. 8. Note the terminal rise in efflux with IAA and NAA following the decline from $\Delta^T \text{max}$.

FIG. 11.

Comparison of basipetal transport of IAA, NAA and 2,4-D in sections from female shoots 22 weeks old. Symbols as in Fig. 8. Note that IAA and NAA effluxes were rising at the termination of the experiment, and the much earlier and greater efflux of 2,4-D in this experiment.

time-delay factor. After attainment of maximum transport ($\Delta T_{\max}$) efflux declined. That age and sex influence the amount of auxin transported is clear from Figs. 6–11. For example, in 3-week male sections $\Delta T_{\max}$ was attained at 3 h (Fig. 7) while in 12,5-week male sections $\Delta T_{\max}$ was not attained until 4,5 h (Fig. 6). Similar differences also appeared between the sexes (e.g. Fig. 10, 11). Wilkins (Personal communication) suggested that the position of $\Delta T_{\max}$ would be influenced also by temperature. However, this would not have been a factor in our experiments as temperature was kept constant at 22°C.

Of major importance is the rapid decline in the amount of radioactivity from the applied auxin after attainment of $\Delta T_{\max}$, which in *Iboza* occurred within 0,5 h of attainment of $\Delta T_{\max}$. This confirms the results of dela Fuente and Leopold (1970 a, b, c) who found that the half-life of the transportable pool of auxin was about 0,5 h in 10-day *Helianthus* seedlings. It is noteworthy that we confirmed their results utilizing quite different techniques.

Toward the end of our investigation we conducted a few preliminary experiments with paper chromatography to examine the possibility that a portion of the applied auxins might be conjugated in the *Iboza* tissues. The results suggested that by seven hours indoleacetyl aspartate (IAAsp) comprised 38 per cent of the radio-activity in the extracts of 12, 5-week stem sections. The formation of IAAsp by the conjugation of exogenous IAA with aspartic acid in peas was reported earlier by Andreae and Good (1957). This and related compounds have also been detected in other plants (Good, Andreae and Van Ysselstein, 1955; Andreae and Van Ysselstein, 1955; Robinson, Forman and Addicott, 1968). In general plant tissues appear to require a two-hour induction period for the formation of IAAsp, with maximal conjugative activity in four to six hours (Andreae and Van Ysselstein, 1960; Zenk, 1964). In *Iboza* maximal conjugation of exogenous auxin appears to coincide with the decline in the transport of the auxin. Chromatograms of NAA-1-¹⁴C and 2,4-D-2-¹⁴C from stem extracts indicated that conjugation of these auxins also takes place in *Iboza*, but as yet the metabolites have not been identified.

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